REMARKS

After entry of the present amendment, claims 1-9, 15-26, 33, 34 and 41-44 are pending in the application. Claims 1 and 18 are herein amended. Claims 32, 35 and 36 are canceled herein. Claims 10-14, 27-31 and 37-40 were canceled in previous amendments. Claims 43 and 44 are added herein.

Claim 1 has been amended to clarify that the sandwich assay method is a quantification method. Basis for this amendment is found on page 22, lines 7-12 and the passage bridging pages 24 and 25. In addition, claim 1 has been amended to more clearly define the meaning of "complex biological fluids." Support for this amendment may be found at least in the canceled claim 32. Moreover, claim 1 has been amended to recite that the engineered protein is "selected from a library constructed by combinatorial means through randomization of a given number of amino acids in a scaffold protein consisting of a naturally occurring protein or domain(s) thereof as a scaffold." Basis for this amendment is found in paragraphs 40-46 of the instant application.

New claims 43 and 44 are added to further specify how the quantification is performed. Basis for these claims is found on page 22, lines 7-12 and the passage bridging pages 24 and 25.

Applicants submit that no new matter is added.

Rejection under 35 USC §112

Claim 18 was rejected under 35 USC §112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner indicated that "*the* B domain" lacks antecedent basis.

Claim 18 is amended to recite "*a* B domain." Accordingly, Applicants submit the rejection has been overcome and respectfully request the Examiner withdraw the rejection.

Rejections under 35 USC §102

Claims 35 and 36 were rejected under 35 USC §102(b) as being anticipated by by Hansson, et al. (Immunotechnol. 4: 237, 1999), or alternatively, International Patent Application No. WO 00/63243 to Ljungqvist, et al.

By this amendment, claims 35 and 36 are canceled thus rendering the rejection moot.

Rejections under 35 USC § 103

The combined teachings of Hansson et al., Cozzette et al. and Self

Claims 1-8, 15-26, 32-36, 41 and 42 were rejected under 35 USC § 103(a) as being unpatentable over the combined teachings of Hansson et al. (Immuntechnol. 4: 237, 1999), Cozzette et al. (US 5,837,446) and Self (US 4,595,655). Applicants respectfully traverse the rejection.

The present invention is directed to a combined use of a non-antibody affinity ligand/a non-natural binder and an antibody or another affinity ligand in a quantification method. It is based on the finding that replacement of one or more antibody by non-antibody affinity ligands in a sandwich assay alleviates or solves the problems with false positive signals in the analysis of samples comprising complex biological fluids. The complex biological fluids may contain antibodies and include serum, plasma, saliva, whole blood, plasma from plasmapheresis, cerebrospinal fluid, amniotic fluid, urine, semen, cord blood, supernatants from cell culture, cell culture media, exsudate and aspirate.

The primary function of the method of the invention is to reduce or completely eliminate cross-linking of the first and second affinity ligands by antibodies present in the sample, in particular heterophilic antibodies (See the paragraph bridging pages 4 and 5).

The method of the invention has the advantage of quantifying very low concentrations of target molecule in a sample (See page 5, lines 18-36). For example, it enables the analysis of small amount of target molecules in samples of high serum concentrations.

Even though Affibody® molecules and other affinity ligands have been known and available for quite some time now, there are still no quantification system similar to the present invention available on the market despite the large demand for such a system. Had the invention been obvious, such a system would have been available a long time ago.

The relied-upon art does not render the instant invention obvious. For example, Hansson et al. made an analysis with BIACORE® which might be described as a capture assay related to the present invention. However, in the analysis, an Affibody® molecule is immobilized to a solid support and then an analyte is added in purified form. Therefore, it is the binding of the analyte that is detected. Thereafter Hansson et al. use a monoclonal antibody against the analyte to determine whether or not it binds to the same epitope as the Affibody® molecule. This step is preformed for the only reason to decide whether or not an Affibody® molecule and an antibody recognize the same analyte. Thus, in contrast with the present invention, Hansson et al. do

not teach or suggest quantification or detection of target molecules in a complex biological fluid.

The Cozzette reference relates to the manufacture of wholly microfabricated biosensors. Even though Cozzette et al. generally disclose that biosensors can be used for sandwich assays (See col. 1, lines 55-57), they do not provide much details of these assays. However, Cozzette et al. do indicate that if a sandwich assay method is used, a biosensor is needed (See col. 47). In view of this teaching, one of ordinary skill in the art would only consider this reference in the context of using a biosensor.

Cozzette et al. recognize the need for the analyses of complex biological fluids, such as whole blood (See the passage bridging columns 2 and 3). However, Cozzette et al. fail to recognize the problems connected with antibodies present in such complex biological fluids, let alone providing a solution to the problem as claimed in the instant application. In fact, Cozzette et al. teach away from the present invention since they point out that the problem connected with such complex biological fluids is that the fluids contaminate the instruments.

Self et al. describe quantification by a coupled enzymatic system, where the presence of an analyte is directly or

indirectly coupled to a measurable enzymatic reaction. The use of receptor pairs as an alternative to an antibody-antibody assay is discussed in the context of obtaining a measurable enzymatic reaction. Self et al. do not teach how to perform a sandwich assay, apart from the coupling to an enzymatic reaction.

The teachings of Self et al. are thus focused on the use of a cyclic chemical reaction for amplification of a determinable product. This is in sharp contrast to the present invention, which enables quantification of a target even in very low concentrations.

Furthermore, Self et al. do not disclose any affinity ligands similar to the ones used in the present invention.

Applicants submit that there is no motivation to combine the teachings of Hansson et al., Cozzette et al. and Self et al. And even if a person skilled in the art did combine the teachings of these references, he would end up with a biosensor based assay for determination whether or not an antibody or an Affibody® molecule binds to a target wherein a cyclic chemical reaction is used in order to amplify the product to be detected. This assay method is not even remotely similar to the sandwich assay method claimed in the instant application.

Furthermore, Applicants submit that there is nothing in the combined teachings of Hasson et al., Cozzette et al. and Self et al. that would lead a person of ordinary skill in the art to the present invention.

It is not possible to pick only information of minor importance from these references without looking at the teachings as a whole, and combine them just in order to fabricate something that would prevent the patenting of the present invention, since this is nothing a person of ordinary skill in the art would ever consider.

Therefore, the present invention is non-obvious over Hansson et al. in view of Cozzette et al. and Self et al. A withdrawal of the rejection is respectfully requested.

The combined teachings of Ljungqvist et al. and Yu et al.

Claims 1-8, 15-26, 32-36, 41 and 42 were rejected under 35 U.S.C. § 103(a) as being unpatentable over the combined teachings of Ljungqvist et al. (WO 00/63243) and Yu et al. (US 6,197,526).

Ljungqvist et al. disclose polypeptides which are derivatives of the B or Z domain of staphylococcal protein A. They do not teach or suggest a sandwich assay for quantification of a target in a complex biological fluid.

Yu et al. teach polypeptides for binding human factor VIII and fragments of human factor VIII and also methods for detecting or purifying human factor VIII and fragments of human factor VIII from a solution such as blood. In col. 15, 1st paragraph, Yu et al. state that it is possible to use a sandwich-type assay. However, there are no experimental data to support this statement. Moreover, Yu et al. fail to teach or suggest quantification of a target. In addition, they did not discuss the problems which are intended to be solved by the present invention, i.e. the problems with false positive signals in analysis of samples comprising complex biological fluids.

There is noting in the combination of Ljungqvist et al. and Yu et al. that would lead one of ordinary skill in the art in the direction of the present invention. The present invention is thus non-obvious over Ljungqvist et al. in view of Yu et al.

The combined teachings of Lin et al., Borrebbaeck et al. and Nygren et al.

Claims 1-9, 15-26, 32-36, 41 and 42 were rejected under 35 U.S.C. § 103 (a) as being unpatentable over the combined teachings of Lin et al. (US 2002/0037506), Borrebaeck et al. (US 2001/0053520), and Nygren et al.(Curr. Opin. Struct. Biol. 7: 463, 1997).

Lin et al. teaches sandwich assays with aptamers for capture and/or detection. The Lin reference, when considered as a whole, focuses on the exclusive use of aptamers - see for example paragraph 0013. Thus, one of ordinary skill in the art would not consider replacing the aptamers with anything else.

Borrebaeck et al. disclose a number of different affinity ligands. However, they only teach the use of these ligands in 2D gel assays. Borrebaeck et al. do not teach or suggest the use of the ligands in capture assays. In view of the strict focus of Lin et al. on aptamers, one of ordinary skill in the art would not replace the aptamers in Lin et al. with affinity ligands disclosed in Borrebaeck et al.

The Nygren reference is a review of different engineered scaffolds and the use thereof for engineering novel binding sites in proteins. In view of the strict focus of Lin et al. on aptamers, one of ordinary skill in the art would not be motivated to combine these references.

Therefore, the present invention is not obvious over Hansson et al in view of Borrebaeck et al. and Nygren et al. Applicants respectfully request the Examiner withdraw the rejection.

Applicants submit that the claims are now in condition for allowance and a prompt Notice of Allowance is respectfully solicited.

If the Examiner believes a telephone conference would aid in the continued prosecution of this application, the Examiner is invited and encouraged to contact Applicants' representative at the telephone number listed below.

Any fees due with this correspondence may be charged to Deposit Account 23-1665 under Customer Number 27267.

Respectfully submitted,

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